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Immobilization of β-Cyclodextrin on Silica Gel**

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β-Cyclodextrin (β-CyD) was covalently immobilized on silica gel by chlorination of silica gel with thionyl chloride or silicon tetrachloride, followed by the reaction of the resulting chlorinated silica gel with β-CyD in pyridine. The amounts of the immobilized β-CyD were 8×10^{-5} to 1.4×10^{-4} mol per 1 g of the β-CyD-silica gel composites. On heating, the composites did not exhibit weight decrease up to 250 °C. The β-CyD in the composites formed 1 : 1 complex with phenol in aqueous solutions, and the equilibrium constant for the dissociation of the complex was 6×10^{-3} mol dm^{-3} at 25 °C. The immobilized β-CyD exhibited catalytic activity in the cleavage of 4-nitrophenyl acetate at 25 °C, pH 8.7.

Cyclodextrins (CyDs), cyclic oligomers of 6—8 glucose molecules, form inclusion complexes with various guest compounds. The magnitude of the complex formation is largely dependent on the structure of guest compound.[1,2] CyDs exhibit specific catalyses in the selective syntheses of various chemicals such as 4-chloroanisole,[3] vitamin K analogs,[4] 4-hydroxybenzaldehydes,[5] 4-hydroxybenzoic acids,[6] and 3-indolecarbaldehyde.[7]

Many works have been carried out on the immobilization of CyDs using organic materials either as cross-linking agents or as supports. Gels, prepared by the reactions of CyDs with epichlorohydrin or diisocyanates, were successfully used for column chromatography[8–13] and selective catalyses.[14–18] CyDs immobilized on polyacrylamide gels as supports were effective for column chromatography.[19] Water-soluble polymers containing CyDs were also prepared.[20]

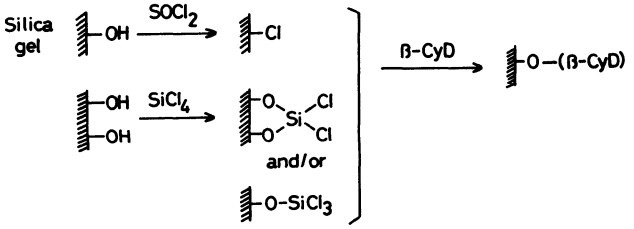
However, there have been a few reports on the immobilization of CyDs on inorganic supports. Fujimura et al.[21] immobilized CyDs to silica gel by the reaction of aminated silica gel with CyD tosylate. Kawaguchi et al.[22] also immobilized CyDs on silica gel by condensation of carboxylated silica gel with CyDs having amino groups by use of 1-ethyl-3-[3-(dimethylamino)propyl]carbodiimide. Both methods require synthesis of modified CyD prior to immobilization.

In this paper, a simpler immobilization of CyDs on silica gel, which does not involve synthesis of modified CyDs, is reported. Silica gel is first chlorinated with thionyl chloride or silicon tetrachloride, and then is reacted with intact β-CyD. Thermal stabilities and binding properties of the β-CyD-silica gel composites are shown. Furthermore, the catalytic activity of the immobilized β-CyD in the cleavage of 4-nitrophenyl acetate is described.

Experimental

Materials. β-CyD was recrystallized from water, and was dried at 110 °C in vacuo. Silica gel (average diameter, 60 μm; surface area, 370 m^2 g^{-1}; average pore diameter, 130 Å; total pore volume, 0.96 dm^3 g^{-1}) was obtained from Iatron Co. and was dried at 110 °C in vacuo. Pyridine was distilled over metallic magnesium ribbon after prolonged refluxing over the same. All other reagents were dried and purified in usual ways.

Immobilization of β-Cyclodextrin on Silica Gel. Immobilization of β-CyD on the silica gel was carried out as depicted in Scheme 1. First, silica gel was added to thionyl chloride or silicon tetrachloride with or without benzene as solvent, and the mixture was refluxed for 5—20 h. The resulting solids were washed with dry benzene, and were dried at 110 °C in vacuo. Then, the solids were reacted with β-CyD in pyridine at 80 °C for 10 h. After the reactions, the solids were repeatedly washed with water, soaked in water for a week, and then dried in vacuo at 110 °C. The contents of β-CyD in the β-CyD-silica gel composites were estimated either from the carbon contents on elemental analyses or from the decrease of weight on thermogravimetric analyses (see Results and Discussion Section). The values determined by these two methods were identical with each other within 1%.



Scheme 1.

Characterization of β-CyD-Silica Gel Composite. Thermogravimetric analyses were made on a Shimadzu Model DT-30 Thermal Analyzer. Surface area (by BET method), pore volume, and pore size (by the Inkley method) were obtained by the analyses of the nitrogen isotherms measured by using an Orr Surface-Area Pore-Volume Analyzer 2100 D (Micromeritics Co.).

Determination of Equilibrium Constant for Complex Formation between β-Cyclodextrin Immobilized on Silica Gel and Phenol. The β-CyD-silica gel composite (100 mg) was added to 3 cm^3 aqueous solution of phenol (the concentration p_0). The mixture was vigorously shaken, and then was incubated at 25 °C for 30 min. The equilibrium concentration (p) of phenol in aqueous medium in the presence of the β-CyD-silica gel composite was determined

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Table 1. Immobilization of β-CyD to Silica Gel

Code	Silica gel	β-CyD	Chlorination of silica gel		Amount of β-CyD in β-CyD–silica gel composite	
	g	g	Agent[a)]	Reaction time/h	wt% in composite	10^{-4} mol per 1 g composite
A-1	18	5	$SOCl_2$(60)	20	16.0	1.4
A-2	18	4	$SOCl_2$(60)	5	10.4	0.88
A-3	18	5	$SOCl_2$/benzene (40/40)	10	15.0	1.3
A-4	20	5	$SiCl_4$/benzene (10/50)	10	9.4	0.83

a) The numbers in parentheses refer to the amounts used (in cm^3).

spectroscopically at 270 nm.

The equilibrium constant K_d for the dissociation of the complex was determined by plotting p vs. $p/(p_0-p)$ according to Eq. 1,

$$p = c_0 \cdot p/(p_0-p) - K_d. \quad (1)$$

Here, c_0 is the amount of the immobilized β-CyD, which is charged per unit volume of specimen. Equation 1 is obtained from Eq. 2, which is based on the assumption that the immobilized β-CyD form 1:1 complex with phenol.

$$K_d = p \cdot [c_0 - (p_0-p)]/(p_0-p). \quad (2)$$

The equilibrium constant for the dissociation of the complex between unmodified β-CyD and phenol in homogeneous solution at 25 °C was determined spectroscopically at 280 nm, according to the method in the literature.[23)]

Kinetics. To a quartz cell, a required amount of β-CyD–silica gel composite and 3 cm^3 of pH 8.7 Tris buffer solution were added at 25 °C. After a thermoequilibrium was attained, 20×10^{-3} cm^3 or less of a stock solution of 4-nitrophenyl acetate in acetonitrile was charged, and the mixture was vigorously stirred. The cleavage was followed by the release of 4-nitrophenol at 400 nm. The rate constants were determined by using usual first-order equation.

Results and Discussion

Immobilization of β-Cyclodextrin on Silica Gel. As shown in Table 1, β-CyD was successfully immobilized on silica gel according to Scheme 1, using either thionyl chloride or silicon tetrachloride as chlorinating agent of silica gel. The amounts of the immobilized β-CyD were 8×10^{-5} to 1.4×10^{-4} mol per 1 g of the β-CyD–silica gel composites. The resulting composites were fine and white particles, as long as purities and dryness of the reagents used, especially of pyridine, were sufficiently high. Otherwise, the particles turned to tan to dark brown color on the treatments.

There were no substantial changes observed either in the scanning electron micrographs of the particles or in the surface area of the particles before and after the treatments.

Figure 1 (a) depicts a thermogravimetric curve for the β-CyD–silica gel composite (code A-1 in Table 1). The composite is stable even at elevated temperatures, and no decomposition is detected up to 250 °C.

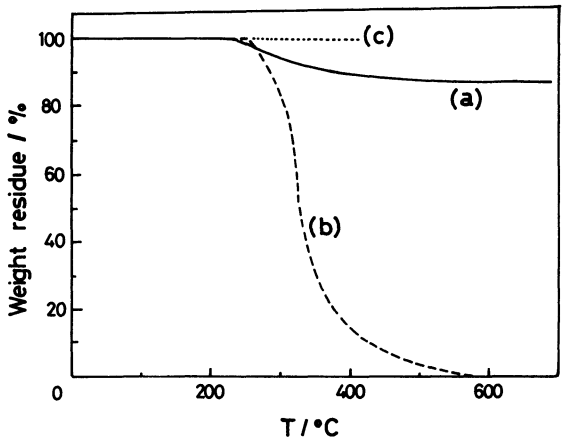

Fig. 1. Thermogravimetric analyses on (a) the β-CyD–silica gel composite (code A-1), (b) β-CyD, and (c) silica gel: the heating rate, 5 °C min^{-1} under air.

Neither color change of the composite nor its softening is observed here. A weight loss due to the oxidative degradation of the β-CyDs in the composites starts at 250 °C and almost ends up at 630 °C, when the heating rate is 5 °C min^{-1}. From the difference between the weight residue at 250 °C and the value at 630 °C, the amount of the β-CyD in the composite is estimated to be 16 wt%. This value satisfactorily agrees with that (15.0 wt%) calculated from the carbon content (6.65%) on elemental analysis.

β-CyD itself starts oxidative degradation at 260 °C, which completes at 580 °C (Fig. 1 (b)).

Virtually identical thermogravimetric curves were obtained for all the other composites A-2, A-3, and A-4, except for the difference in the magnitude of weight loss due to the difference in the amount of the β-CyD immobilized on the silica gel.

Covalent attachment of the β-CyD to silica gel was confirmed by the absence of loss of the β-CyD moieties on repeated washing of the composites with water or 0.002 M hydrochloric acid (1 M=1 mol dm^{-3}). The amount of the β-CyD was determined by thermogravimetric analyses on these specimens.

The largest value for the immobilization of β-CyD (1.4×10^{-4} mol per 1 g of the composite, code A-1) corresponds to occupation of 380 A^2 surface of the silica gel by one β-CyD molecule: the silica gel has surface

Table 2. Equilibrium Constant K_d for the Dissociation of the Complex between the β-CyD Immobilized to Silica Gel and Phenol in Aqueous Solution at 25°C

β-CyD–silica gel composite	K_d	Amount of β-CyD/10^{-6} mol[a)]	
	10^{-3} mol dm^{-3}	Charged	Kinetically determined
A-2	6.5±1.9	8.8	7.3±2.0
A-4	6.6±0.8	8.3	8.0±0.6
Intact β-CyD (Non-immobilized)	1.6±0.3	—	—

a) Amount of β-CyD in 3 cm^3 for specimen.

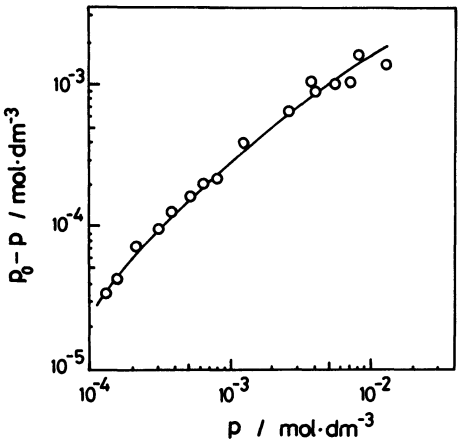

Fig. 2. Plot of (p_0-p) vs. p for the complex formation between the β-CyD immobilized on silica gel and phenol in aqueous solution at 25°C: 100 mg of the composite A-2, which contained 8.8×10^{-6} mol of β-CyD, was added to 3 cm^3 of phenol solution with the charged concentration p_0, and the equilibrium concentration p of phenol in aqueous medium was measured.

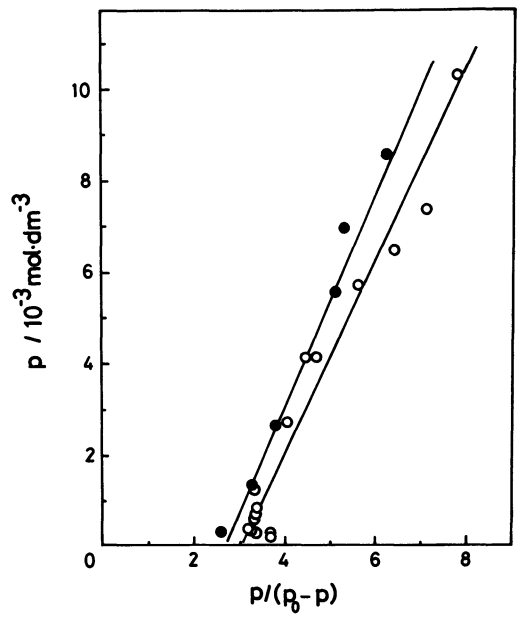

Fig. 3. Plots of p vs. $p/(p_0-p)$ for the complex formation between the β-CyD immobilized on silica gel and phenol in aqueous solution at 25°C: ○, composite A-2 (the charged amount of β-CyD, 8.8×10^{-6} mol); ●, the composite A-4 (the charged amount of β-CyD, 8.3×10^{-6} mol); Definition of p and p_0 as well as the experimental methods should be referred to the footnote in Fig. 2.

area of 370 m^2 per 1 g. This value is only 2.1 fold larger than the molecular cross-section area (184 A^2) of β-CyD, perpendicular to the longitudinal axis of the cavity. The outer diameter of β-CyD is 15.3 A.[2] Thus, the surface of the silica gel is largely covered by β-CyD molecules in the composites.

Complex Formation between the β-Cyclodextrin Immobilized on Silica Gel and Phenol. When the β-CyD–silica gel composite was mixed with aqueous phenol solution and the mixture was vigorously shaken, the concentration of phenol in the aqueous medium rapidly decreased. This shows that the β-CyD immobilized to silica gel forms complex with phenol and takes it out of the aqueous medium. Usually equilibrium was attained in less than 20 min.

Figure 2 depicts the plot of (p_0-p) vs. p. Here, 100 mg of the composite A-2, which contained 8.8×10^{-6} mol of β-CyD, was added to 3 cm^3 of aqueous phenol solution of the charged concentration p_0, and the equilibrium concentration p of phenol in the aqueous medium was measured. Logarithm of (p_0-p), which corresponds to the amount of phenol forming complex with the immobilized β-CyD, asymptotically increases with logarithm of p, the equilibrium concentration of phenol in the solution.

In Fig. 3, p is plotted against $p/(p_0-p)$ according to Eq. 1 for the complex formation of phenol with the immobilized β-CyDs in the composites A-2 and A-4. For both of the composites, linearities of the plots are satisfactorily fine. These results confirm that the β-CyDs immobilized on the silica gel form 1:1 complexes with phenol. Cooperativity of two immobilized β-CyD molecules or more in the complex formation is not detected.

Table 2 lists the equilibrium constants K_d for the dissociation of the complex between the β-CyD immobilized on silica gel and phenol in aqueous solution at 25°C. The K_d values for the immobilized β-CyDs in both of the composites A-2 and A-4, determined from the intercepts on the ordinate (see Eq. 1), are almost identical with each other. About 4 fold larger K_d values for these immobilized β-CyDs, compared with the value for intact β-CyD, are associated with larger steric hindrance on the complex formation.

Table 3. Catalytic Activity of the β-CyD Immobilized to Silica Gel in the Cleavage of 4-Nitrophenyl Acetate[a)]

Catalyst	Amount of β-CyD/10^{-6} mol	Pseudo-first order rate constant of cleavage/10^{-4} s^{-1}
β-CyD–silica gel composite		
A-2 (100 mg)	8.8	1.5
A-3 (60 mg)	7.8	1.9
A-4 (100 mg)	8.3	1.9
A-4 (300 mg)	24.9	2.4
Intact β-CyD (9.4 mg)	8.3	2.9
(Non-immobilized) (11.2 mg)	9.9	3.2
(Non-immobilized) (28.3 mg)	24.9	5.8
Silica gel (100 mg)	0.0	1.2
None	0.0	1.2

a) Catalysts were added to 3 cm^3 of pH 8.7 Tris buffer solution. The charged concentration of 4-nitrophenyl acetate was 3.3×10^{-5} mol dm^{-3}.

The amounts of the immobilized β-CyD in the specimens, determined from the slopes of the straight lines in Fig. 3 are in good agreements with the charged amounts, estimated from the compositions of the β-CyD–silica gel composites. This fact shows that β-CyDs were successfully immobilized on the silica gel in their intact forms and also confirms the validity of the procedures employed in the present study for the determination of K_d values.

Catalytic Activity of the β-Cyclodextrin Immobilized on Silica Gel. The cleavage of 4-nitrophenyl acetate in the presence of the β-CyD–silica gel composites obeyed first-order kinetics. This result rules out a possibility that diffusion of the substrate to the vicinity of the immobilized β-CyD is rate-determining.

As shown in Table 3, all the β-CyD–silica gel composites exhibit catalytic activities for the cleavage of 4-nitrophenyl acetate. The activity is definitely attributed to the functions of the immobilized β-CyD, since the silica gel alone has no catalytic activity at all.

The catalytic activities of the immobilized β-CyDs are 2—4 fold smaller than that of intact β-CyD. This is associated with smaller substrate-binding constant, as shown in Table 2. Kinetic determination of the catalytic rate constants and the substrate binding constants was not successful, due to small magnitude of acceleration.

Conclusion

β-Cyclodextrin was covalently immobilized on silica gel as support. The resulting composites were stable up to 250 °C, where most of organic supports or organic polymers were hard to be used due to decomposition or deformation. The immobilized β-cyclodextrins exhibited both binding and catalytic activities.

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